

3296-Pos Board B451**Assembly and Architecture of Gram-Positive and -Negative Cell Walls**James C. Gumbart¹, Morgan Beeby², Grant Jensen³, Benoit Roux⁴.¹Georgia Tech, Atlanta, GA, USA, ²Imperial College, London, United Kingdom, ³Caltech, Pasadena, CA, USA, ⁴U. Chicago, Chicago, IL, USA.

The cell wall, a porous mesh-like structure, provides shape and physical protection for bacteria. At the atomic level, it is composed of peptidoglycan (PG), a polymer of stiff glycan strands cross-linked by short, flexible peptides. However, at the mesoscale, multiple models for the organization of PG have been put forth, distinguished by glycan strands parallel to the cell surface (the so-called "layered" model) or perpendicular (the "scaffold" model). To test these models, and to resolve the mechanical properties of PG, we have built and simulated at an atomic scale patches of both Gram-positive and negative cell walls in different organizations up to 50 nanometers in size. In the case of Gram-positive PG, molecular dynamics simulations of the layered model are found to elucidate the mechanisms behind a distinct curling effect observed in three-dimensional electron cryo-tomography images of fragmented cell walls. For Gram-negative PG, simulations of patches with different average-glycan-strand lengths reveal an anisotropic elasticity, in good agreement with atomic-force microscopy experiments. Insights from the simulations reveal how mesoscopic and macroscopic properties of a ubiquitous bacterial ultrastructure arise from its atomic-scale interactions and organization.

3297-Pos Board B452**Cell Shape Can Mediate the Spatial Organization of the Bacterial Cytoskeleton**Siyuan Wang¹, Ned S. Wingreen².¹Harvard University, Cambridge, MA, USA, ²Princeton University, Princeton, NJ, USA.

The bacterial cytoskeleton guides the synthesis of cell wall and thus regulates cell shape. Since spatial patterning of the bacterial cytoskeleton is critical to the proper control of cell shape, it is important to ask how the cytoskeleton spatially self-organizes in the first place. In this work, we develop a quantitative model to account for the various spatial patterns adopted by bacterial cytoskeletal proteins, especially the orientation and length of cytoskeletal filaments such as FtsZ and MreB in rod-shaped cells. We show that the combined mechanical energy of membrane bending, membrane pinning, and filament bending of a membrane-attached cytoskeletal filament can be sufficient to prescribe orientation, e.g. circumferential for FtsZ or helical for MreB, with the accuracy of orientation increasing with the length of the cytoskeletal filament. Moreover, the mechanical energy can compete with the chemical energy of cytoskeletal polymerization to regulate filament length. Notably, we predict a conformational transition with increasing polymer length from smoothly curved to end-bent polymers. Finally, the mechanical energy also results in a mutual attraction among polymers on the same membrane, which could facilitate tight polymer spacing or bundling. The predictions of the model can be verified through genetic, microscopic, and microfluidic approaches.

Unconventional Myosins**3298-Pos Board B453****Force Generation by Membrane-Bound Myo1c, a Single Molecule Study**

Serapion Pyrpasopoulos, Henry Shuman, E. Michael Ostap.

University of Pennsylvania, Philadelphia, PA, USA.

Myosin-1C (Myo1c) is a class-I myosin that links cell membranes to the actin cytoskeleton. Actin binding occurs via the motor domain, while the tail domain interacts with phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) through a pleckstrin homology domain. We expressed recombinant full-length myo1c and we showed previously that it is able to propel actin filaments while attached to a fluid supported-lipid-bilayer containing PtdIns(4,5)P₂ in an in vitro gliding assay. However, Myo1c in this ensemble assay undergoes its working stroke under very low-loads, and it is of interest to measure the ability of the motor to generate force while bound to a fluid bilayer. Therefore, we measured the working stroke of membrane-bound myo1c molecules using an optical tweezers in the three-bead assay configuration. We engineered spherical supported bilayers as pedestal beads with membranes consisting of 98% DOPC and 2% PtdIns(4,5)P₂. Membrane fluidity of the bilayers was confirmed by FRAP of labeled lipids. We detected actin attachments of single membrane-bound Myo1c molecules. We observed unitary work stroke events against the force of the trap, followed by the relaxation of tension presumably due to diffusion of the Myo1c on the lipid bilayer. The size of the working stroke of membrane-bound Myo1c was reduced compared to Myo1c on a solid sub-

strate, possibly due to the elasticity of the lipid membrane. The subsequent relaxation of the tension takes place within 100 ms of the working stroke. Our experimental results show that Myo1c can develop tension on fluid membranes and may therefore actively contribute to tension between the membrane and actin, as well as to powering changes in membrane morphology. This assay will be useful to investigate the mechanical properties of the large number of cytoskeletal proteins that are proposed to link membranes and actin. Supported by NIH RO1GM57247.

3299-Pos Board B454**Exploring the Force-Sensitivity of Acanthamoeba Myosin 1C Function**

Jay Howard, David Altman.

Willamette University, Salem, OR, USA.

Myosin motors have the ability to convert the chemical energy associated with ATP hydrolysis into mechanical energy, allowing them to do work within the cell. Each of the various myosin classes performs different tasks, ranging from assisting in locomotion of the cell to trafficking cargo within the cell. It has been hypothesized that class 1 myosins fit into two subclasses: subclass-2 myosins, which are thought to have a force-dependence; and subclass-1, which are thought not to. While a functional response to external forces has been observed for a subclass-2 myosin 1 (rat myosin 1b), a force-sensitivity in subclass-1 myosins has not yet been explored. We examined the force-sensitivity of Acanthamoeba myosin 1c (AM1C) activity, a subclass-1 myosin 1. Here we present the results of single molecule optical trap studies of the activity of AM1C when experiencing forces opposing its direction of motion.

3300-Pos Board B455**Kinetic Characterization of Nonmuscle Myosin IIB at the Single Molecule Level**

Attila Nagy, Yasuharu Takagi, Neil Billington, Earl Homsher,

James R. Sellers.

NIH, Bethesda, MD, USA.

Nonmuscle myosin IIB (NMIIB) is a cytoplasmic myosin, which plays an important role in cell motility by maintaining cortical tension. It forms bipolar thick filaments with ~14 myosin molecule dimers on each side of the bare zone. Our previous studies showed that the NMIIB is a moderately high duty ratio (~20-25%) motor. The ADP release step (~0.35 s⁻¹), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 ± 0.01 s⁻¹), and as a result acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily (<0.15 μM). The aim of this study was to relate the known in vitro kinetic parameters to the results of single molecule experiments and to compare the kinetic and mechanical properties of a single- and double-headed myosin fragments, and nonmuscle IIB thick filaments. Examination of the kinetics of NMIIB interaction with actin at the single molecule level was accomplished by the use of TIRF using FIONA and dual-beam optical trapping. At a physiological ATP concentration (1 mM), the rate of detachment of the single-headed and double-headed molecules was similar (~0.4 s⁻¹). Using optical tweezers we found that the power-stroke sizes of single- and double-headed HMM were ~6 nm. No signs of processive stepping at the single molecule level were observed in the case of NMIIB-HMM in optical tweezers or TIRF/in vitro motility experiments. In contrast robust motility of individual fluorescently labeled thick filaments of full-length NMIIB was observed on actin filaments. Our results are in good agreement with the previous steady state and transient kinetic studies and show that the individual nonprocessive non-muscle myosin IIB molecules form a highly processive unit when polymerized into filaments.

3301-Pos Board B456**Magnesium-Dependence and Active Site Dynamics in Myosin V and Myosin II**Anja M. Swenson¹, Joseph M. Muretta², Faith D' Amico¹,William C. Unrath¹, David D. Thomas², Christopher M. Yengo¹.¹Pennsylvania State University College of Medicine, Hershey, PA, USA,²University of Minnesota, Minneapolis, MN, USA.

We examined the magnesium-dependence of heavy meromyosin V (MV HMM) and two class II muscle myosins, skeletal muscle myosin (SK HMM) and smooth muscle myosin (SM HMM), in the in vitro motility and solution actin-activated ATPase assays. We find that all three myosins are inhibited in a Mg²⁺-dependent manner (0.5-10 mM MgCl₂) in both ATPase and motility assays, under conditions in which the ionic strength was held constant. Interestingly, MV was more steeply dependent on magnesium than both SK and SM. In addition, actin filament breaking in the motility assay was two-fold faster with MV HMM at high (10 mM) compared to low (1 mM) MgCl₂ conditions. To begin to understand how the active site of myosin V is different from myosin II we examined the time resolved fluorescence